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DECEMBER 1991

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ADMINISTRATIVE REPORT LJ-91-44C

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VALIDATION OF BIOELECTRICAL IMPEDANCE ANALYSIS FOR ASSESSING BODY COMPOSITION OF CALIFORNIA SEA LIONS

A REPORT TO NATIONAL MARINE FISHERIES SERVICE

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September 1991

In partial fulfillment of contract number 40-JGNF-0298

SWFS ADMINISTRATIVE REPORT LJ-91-44C

INTRODUCTION

As marine mammal populations numbers increase, the need for objective criteria to describe both animal and habitat condition will also increase. The literature on mammalian population dynamics often makes vague reference to the concept of population condition. This term usually refers to an animal's chances of living or dying, and it can be indexed by a variety of factors, including deposited fat reserves, blood chemistry, and aspects of body growth (e.g., Ratcliffe 1980; Leader-Williams and Ricketts 1982; DelGiudice *et al.*, 1987; Barber and Coblentz 1987; Servello and Kirkpatrick 1987). Most mammals exhibit seasonal changes in body condition related to variation in either ambient temperature or food quality or quantity. These changes are most frequently exhibited as changes in total body fat.

Body condition can tell a lot about how well a population is responding to its environment. If a population is healthy and expanding, e.g., California sea lions (*Zalophus californianus*) or northern elephant seals (*Mirounga angustirostris*), it is expected to be in good condition. If, on the other hand, a population has exceeded the carrying capacity of its environment, body condition would be expected to decline. Similarly, in years of poor food availability, e.g. El Niño years, body condition would also decline. Measures of body condition can therefore be a useful index for managers to monitor environmental quality and perhaps, indirectly, population size.

Probably the most important measure of body condition involves the determination of total body fat. There is no simple method of directly measuring body fat of animals in the field and therefore it is usually measured indirectly. One of the most commonly employed techniques is the injection of an accurately known volume of tritiated water (e.g., Costa 1987), but this technique requires an equilibrium period of several hours.

The tritium dilution technique is based on the premise that the accurately known amount of tritium which is injected into the animal equilibrates throughout the total body water pool of the animal. Given that there is a known amount of tritium injected into the animal, the degree to which it was diluted can be accurately measured, and the total volume of water can be calculated. Once the total body water pool (TBW) is known, fat-free mass (FFM) is calculated by assuming a hydration state of 73% (Pace and Rathbun 1945). The difference between FFM and total mass is fat mass. The tritium technique

has been compared to carcass compositions determined by homogenizing the whole animal and compares well with direct analyses in a wide variety of species (e.g., Pace and Rathbun, 1945; Foy and Schneiden, 1960; Crum *et al.*, 1985; Reilly and Fedak, 1990).

There are additional technologies available to determine fat content of these animals. Ultrasound is commonly used to estimate blubber depth, but recently it has also been used to estimate blubber mass by utilizing measures of blubber depth, girth and length from the end of the body to each of the girth rings (Gales and Burton 1987; Worthy *et al.* in press). This approach utilizes truncated cones to estimate blubber volume and then, by accounting for blubber density, a blubber mass can be calculated.

Another approach is to use Bioelectrical Impedance Analysis (BIA). BIA is a localized 80 μ amp, 50-kHz current-injected system that yields a measure of total body resistivity, the inverse of conductivity. The BIA method is based on the conduction of an applied electrical current in the organism and is essentially instantaneous. Biological impedance measurements have been related to biological functions such as pulsatile blood flow (Nyboer 1970) and to the determination of total body water in healthy and diseased humans (e.g., Hoffer *et al.* 1969, Lukaski *et al.* 1985). Lukaski *et al.* (1985) established a strong relationship between conductance and fat-free mass by utilizing the idea that fat-free mass has a much greater conductivity than fat does.

The purpose of the present study was to validate the application of Bioelectrical Impedance Analysis to California sea lions by comparing it with estimates of total body water derived using tritiated water methodology. Secondly, I compared estimates of blubber mass to our estimates of total body fat to determine the distribution of total body fat stores between insulation and energetic stores.

MATERIALS AND METHODS

California sea lions (*Zalophus californianus*) were captured on San Nicolas Island (SNI) April 20-22, 1990 (n=23) and April 19-22, 1991 (n=18). All animals were given an initial injection of 1.0 mCi of tritiated water (HTO) and held in a holding cage for a 3 h equilibrium period. At the end of 3 h, animals were weighed and standard length and axillary girth were measured.

Determinations of resistance were made using a tetrapolar impedance plethysmograph or Bioelectrical Impedance Analysis (BIA) system (model BIA-101A, RJL Systems, Detroit MI). The tetrapolar method was used to minimize contact impedance or skin-electrode interaction (Hoffer *et al.* 1969, Lukaski *et al.* 1986). Animals were manually restrained on a restraint board and two pairs of electrodes were positioned. Posterior leads were clipped 2 cm from the tip of the trailing edge of the hind flipper with an approximately 2 cm separation between the two leads. Anterior leads were connected to 22 ga stainless steel needles which were inserted subcutaneously on the top of the head. Needles were necessary to position the electrodes subcutaneously and thereby "inject" the current, bypassing the electrically insulative blubber layer. A small quantity of electrolyte gel was applied in each case to ensure good skin contact. An excitation current of 80 μ amp at 50 kHz was introduced at the distal electrodes of the head and flipper. A reading was determined to be stable when values were constant for a minimum of 10 seconds.

Blubber depths were measured using an ultrasound device accurate to within $\pm$ 0.5 mm (Scanoprobe II, Scano, Ithaca, New York) at dorsal and lateral sites around girth rings located at the neck, axilla, umbilicus, and pelvis. Total body length and the distance between the tip of the tail and each of these rings was also measured. Animals were then individually tagged and allowed to go free.

Sample Analysis and Calculations

Water was diluted from blood samples (Ortiz *et al.*, 1978; Costa *et al.*, 1986; Costa, 1987) and specific activity determined in triplicate by scintillation spectrophotometry of 100 μ l of distilled water in 10 ml of Betaphase cocktail (Westchem, San Diego, CA). TBW volume was determined from the dilution of tritium in the body (Foy and Schneiden, 1960) and corrected for the approximately 4% overestimate in TBW (Crum *et al.*, 1985; Reilly and Fedak, 1990). Using TBW volume, fat-free mass (FFM) was calculated assuming an average hydration state of 73% (Pace and Rathbun, 1945; Ortiz *et al.*, 1978). Fat mass was calculated as the difference between total body mass and FFM.

Blubber volume was estimated using the method of Gales and Burton (1987) as modified by Worthy *et al.* (in press), wherein a series of interlocking truncated cones were used to convert length, girth and blubber depth data to a blubber volume. This volume was converted to mass assuming a blubber density of 0.94 g cm^{-3} (Gales and Burton, 1987; G.A.J. Worthy, unpubl. data). This technique presumes that subcutaneous fat stored in the flippers and on top of the head is insignificant.

Equations describing the relationship between the BIA resistance (R) and reactance (Xc) values and total body water (determined from tritium dilutions) were derived using best subset and stepwise linear regression (Statistix, Analytical Software). This relationship included terms describing length, axillary girth, mass and various combination of these variables.

RESULTS

California sea lions handled in the present study ranged in mass from 25-105 kg and in length from 105-180 cm (Fig. 1). The sex ratio of animals less than 50 kg was 9 females to 10 males, whereas adults (over 60 kg) were almost exclusively females ($n=21$), with only 1 male being handled. The sex ratio of adult animals is indicative of the distribution pattern of animals on the beach during the non-breeding season.

Lateral axillary blubber depths of adult sea lions did not differ between 1990 and 1991 (Fig. 2). Blubber depth measurements of the young-of-the-year animals suggested thinner blubber depths in 1991 as compared to 1990 (Fig. 2). When blubber depths and girth measurements were used to calculate blubber mass, inter-annual difference disappeared (Fig. 3). Young-of-the-year animals showed blubber mass estimates ranging from 14-22% of total mass (Fig. 3). Adult sea lions exhibited blubber masses ranging from 11-22% of total mass (Fig. 3).

Total body fat estimates derived from tritiated water dilution indicated a lack of inter-annual differences for adult sea lions (Fig. 4). Results for the young-of-the-year animals (Fig. 4) were consistent with the suggestion of a lower body condition on 1991 which was implied by the depth data (Fig. 3).

A comparison of calculated values of blubber mass as a percentage of total mass compared to total body fat derived from tritiated water dilution indicated that in adult sea lions these two values are very similar (Fig. 5). Young-of-the-year sea lions, however, appear to have internal stores of body fat in addition to those found in the blubber layer (Fig. 5). Total fat stores of pups ranged from 25-37% of total body mass (Fig. 5).

The relationship between resistance and reactance and total body water volume is a function of the shape of the animal. Specifically the inter-electrode distance (estimated by the total body length) and the cross-sectional area (estimated by axillary girth) are important terms. A relationship was derived between total body water, estimated by tritiated water dilution, and terms including resistance (R), reactance (Xc), length (L), axillary girth (G), mass (M), L^2/R , L^3/R , Xc^3/R , Xc^3/R , M/R and M/Xc . Best subset and reverse stepwise regression analysis resulted in a relationship containing length (L), axillary girth (G), mass (M), L^3/R , and M/R . Total body water (TBW) was best estimated by:

$$TBW = (158.46 \times (M/R)) + (0.94 \times L) - (0.20 \times G) - (0.003 \times (L^3/R)) - 82.33$$

$$R^2 = 0.90, n=38$$

A comparison of BIA calculated total body fat and blubber mass (Fig. 6) calculated from girth and blubber depth data show a similar relationship as did Fig. 5. The data in Fig. 6 show a greater variance in BIA-measured fat, particularly in younger animals.

The direct relationship between resistance (Fig. 7) or reactance (Fig. 8) and TBW was weak. The Pearson correlation between R and TBW was -0.3122 and between Xc and TBW was -0.1883. The best Pearson correlations were actually between TBW and L (0.9451), TBW and M (0.9395) and TBW and G (0.7634). The relationship between resistance and/or reactance and TBW is not a simple one, however in human applications the relationship is much stronger.

The equation which has resulted from this study is driven primarily by morphometrics and not by either resistance or reactance. The substitution of widely varying resistance values into the equation did not result in radically different TBW estimates, suggesting minimal importance of the resistance term (Table 1). Relatively

minor changes in length or girth result in comparable changes in the estimate of TBW (Table 2). This suggests a potential problem with current injection.

DISCUSSION

Bioelectrical Impedance Analysis is a technique which has achieved widespread application in the human health and fitness fields. The technique is based on the premise that the resistance of the body to an applied electrical current is proportional to the total body water volume. This technique is sensitive to a variety of factors, including position of the electrodes, orientation of the limbs and position of the body, as well as how much time has passed since drinking or feeding. Postural changes are limited when working with marine mammals, however, the injection of current into the body is critical. The results of the present study suggest that this may be a problem.

I believe that the observed lack of robustness of the BIA model is due to complication associated with the attachment technique. In any given measurement, stability was achieved. This is usually indicative of good contact having been attained with all electrodes. The minimal importance of the resistance term in equation [1] suggests that there may be variability associated with the effectiveness of the connection, likely with the rear flippers. This suggests that a modification of the attachment technique is required. This potential problem is currently being investigated by utilizing captive phocids at Memorial University of Newfoundland where there is the opportunity for obtaining repeated measures over a restricted time period. Results of this independent study confirms that repeatable, stable readings of resistance can be obtained by utilizing needles positioned subcutaneously on both the head and ankles (G.A.J. Worthy, R. Gales and D. Renouf, unpubl. data). This repeatability suggests that a more appropriate regression equation can be developed using these new data.

It is interesting that the derived relationship is as powerful as it is. This is reminiscent of previously described condition indices which utilize ratios of mass, length and girth and suggests the possibility that body condition could be estimated using some easily derived external measurements.

Gales and Burton (1987) estimated total subcutaneous blubber volume, and indirectly blubber mass, in southern elephant seals by using blubber depth measurements, obtained using ultrasound at 18 body sites, together with circumference and length measurements. Worthy *et al.* (in press) used ultrasound in a similar manner to monitor declines in energy stores during the molt fast of adult female northern elephant seals. Ultrasound is a safe, non-invasive and logistically practical technique, which has been used on a wide variety of pinniped species to measure blubber depth (*i.e.*, Worthy and Lavigne, 1983; 1987; Gales and Burton, 1987; Worthy *et al.*, in press). The utilization of blubber depth to estimate blubber mass is easy to perform and the accuracy of the technique appears to be good (Gales and Burton, 1987; Ryg *et al.*, 1990; Worthy *et al.*, in press), but it only gives information on fat stored in the hypodermis and not on total body fat stores. In some species, such as the grey seal, it would give a reasonable estimate of total fat stores (Worthy and Levine, 1987). Apparently, this is also the case with California sea lions. Young-of-the-year sea lions are similar to harp seals, while adult sea lions greatly resemble grey seals.

Tritiated water is commonly used to measure body water and allows for the estimation of total body fat. Potential errors associated with this technique have been discussed previously and relate primarily to errors associated with the assumption of hydration state and routes of water loss (see Costa, 1987 for a review). Comparison of fat estimates derived using tritiated water and ultrasound suggests that virtually all of the body fat stores in the northern elephant seal are in the hypodermis (Worthy *et al.*, in press).

Both ultrasound and tritiated water techniques have errors associated with them. The advantage of using the two together is the capability of separating the total body fat stores into those in the hypodermis (insulation) and those which are stored elsewhere in the body (energy stores).

The results of the present study suggests that the manner in which the BIA leads were attached resulted in inadequate current injection and derived resistance values were not a useful predictor of TBW. The system still has a great deal of promise and further study is warranted.

ACKNOWLEDGEMENTS

I thank the very capable people who aided in the field captures and manipulations, especially D. Costa, D. Crocker, J. Liao, J. Sanders, K. Rossbach, P. Thorson, and J. Williams. I also thank R. Dow, G. Smith, M. Klope and all the rest of the staff in the Environmental Division and the commands at SNI/OLF and PMTC of Pt. Mugu Naval Air Station for accommodating my research at San Nicolas Island. Distillations were done by V. Kirby, M. Kretzmann, L. Rea and J. Williams. This research was authorized under National Marine Fisheries Service permit numbers 422 and 721 issued to D.P. Costa and G.A.J. Worthy. Jay Barlow served as Contracting Officer's Technical Representative (COTR) for this contract.

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FIGURE LEGEND

- Figure 1: Length-mass relationships for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 2: Lateral axillary blubber depths for California sea lions handled on San Nicolas Island in April 1990 and 1991.
- Figure 3: Blubber mass calculated from girth and blubber depth data as a percentage of total body mass for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 4: Total body water estimates derived from tritiated water dilution space for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 5: Total body fat (calculated by using tritiated water) compared to blubber mass (calculated from girth and blubber depth data) for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 6: Total body fat (calculated by using BIA) compared to blubber mass (calculated from girth and blubber depth data) for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 7: Resistance as a function of total body water (calculated from tritiated water dilution) for California sea lions handled on San Nicolas Island in April of 1990 and 1991.
- Figure 8: Reactance as a function of total body water (calculated from tritiated water dilution) for California sea lions handled on San Nicolas Island in April of 1990 and 1991.

Table 1: Estimates of TBW derived using equation [1] assuming an animal with a mass of 100 kg, length of 180 cm, and an axillary girth of 120 cm. Resistance values were varied.

Resistance (ohms)	Total Body Water (l)
100	58.04
200	60.46
300	61.26
400	61.66
500	61.91
600	62.07
700	62.18
800	62.27
900	62.34

Table 2: The effect of varying length (cm) and girth (cm) measurements while maintaining mass (kg) and resistance (ohms) values.

L	G	M	R	TBW
175	115	100	400	58.59
180	115	100	400	59.74
185	115	100	400	60.70
175	120	100	400	57.59
180	120	100	400	58.74
185	120	100	400	59.70
175	125	100	400	56.59
180	125	100	400	57.74
185	125	100	400	58.70

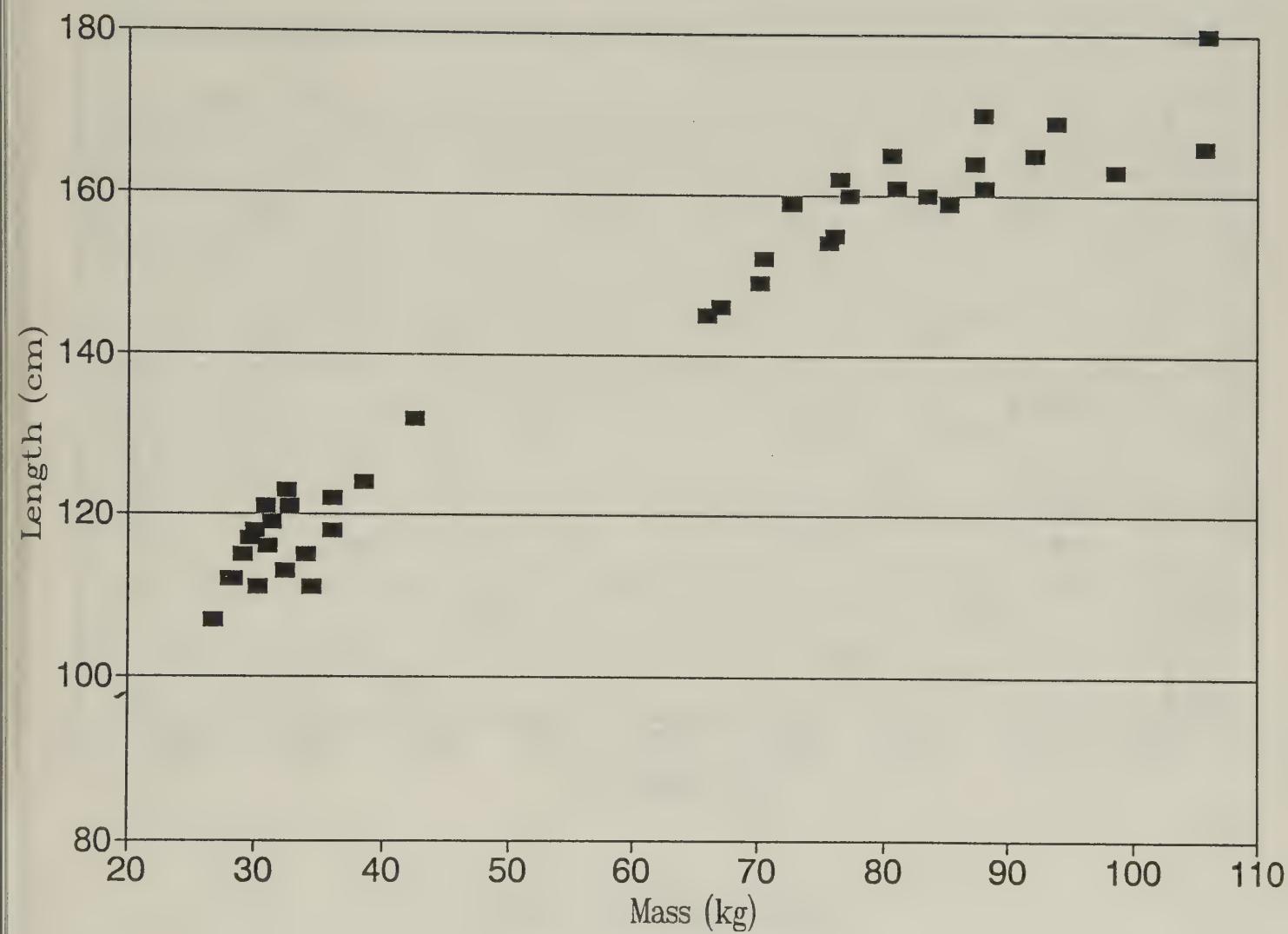


Figure 1: Length-mass relationships for California sea lions handled on San Nicolas Island in April of 1990 and 1991.

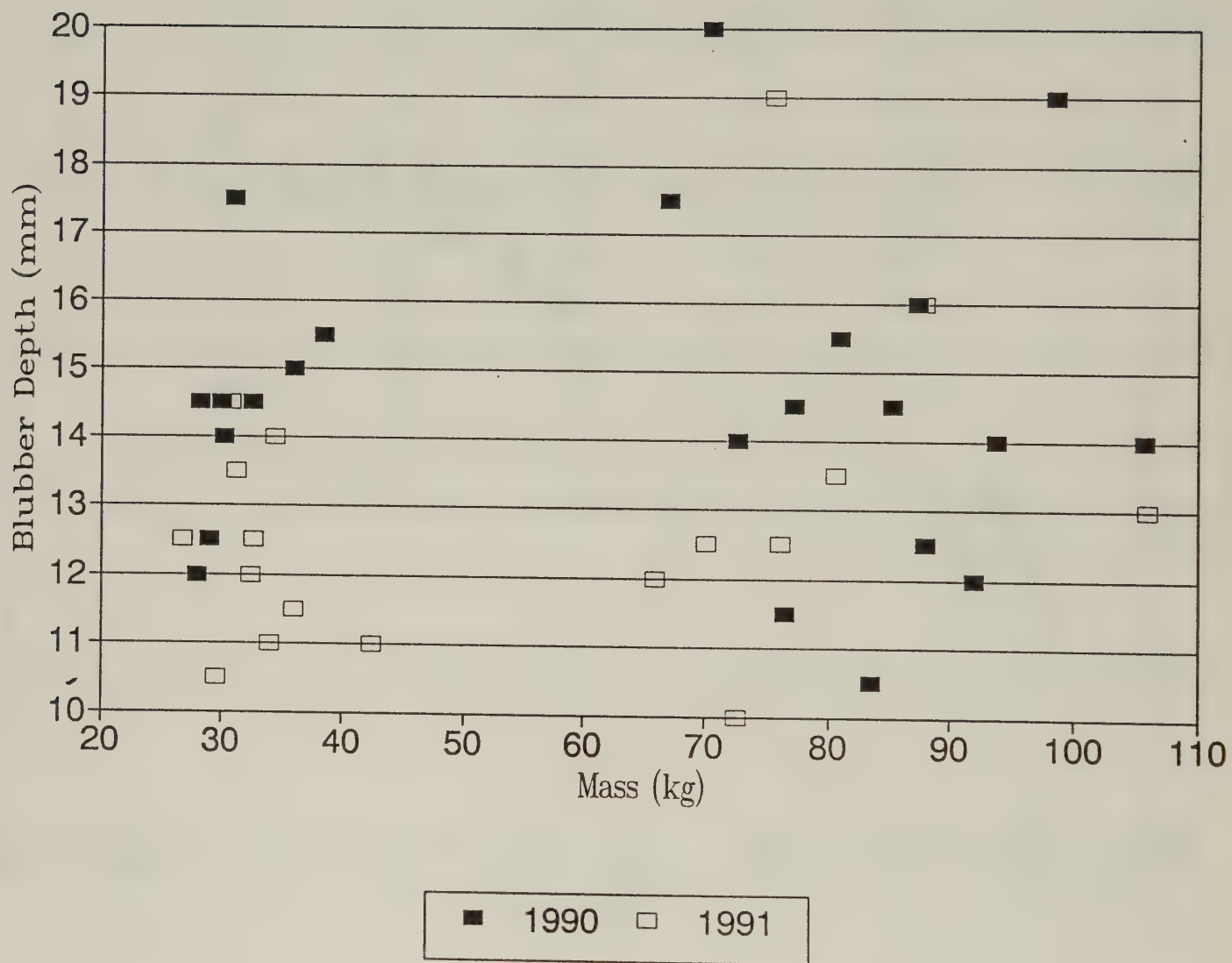


Figure 2: Lateral axillary blubber depths for California sea lions handled on San Nicolas Island in April 1990 and 1991.

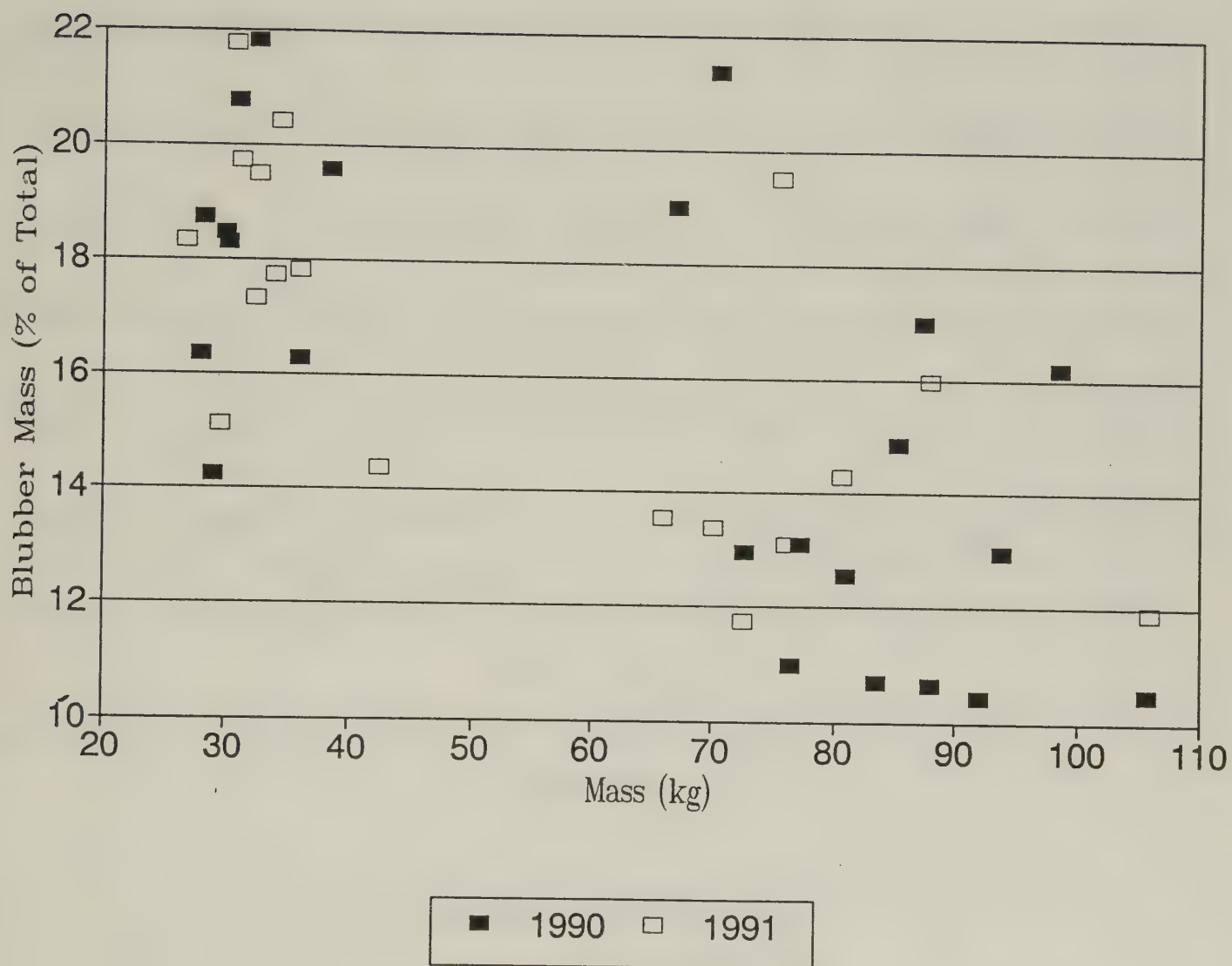


Figure 3: Blubber mass calculated from girth and blubber depth data as a percentage of total body mass for California sea lions handled on San Nicolas Island in April 1990 and 1991.

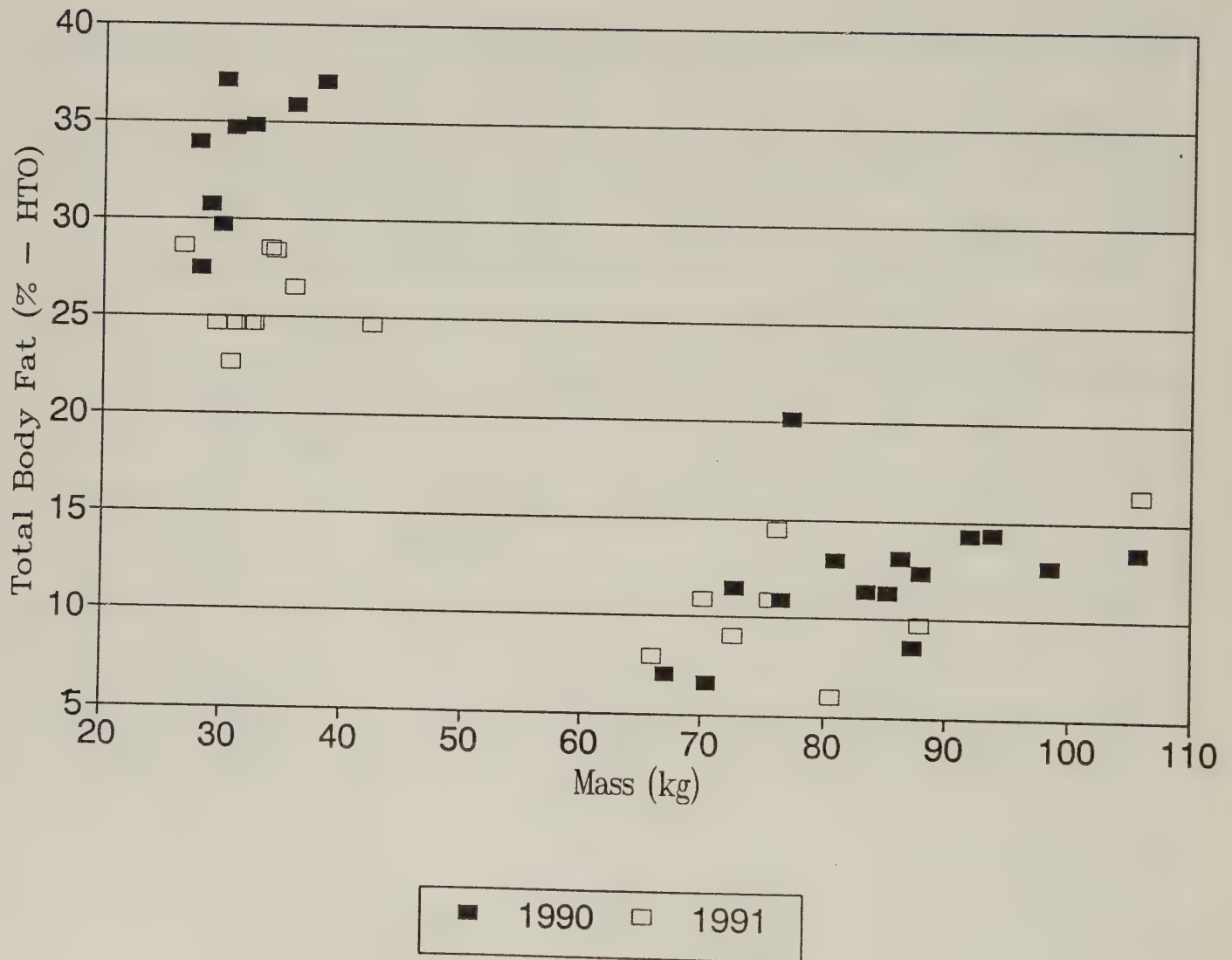


Figure 4: Total body water estimates derived from tritiated water dilution space for California sea lions handled on San Nicolas Island in April 1990 and 1991.

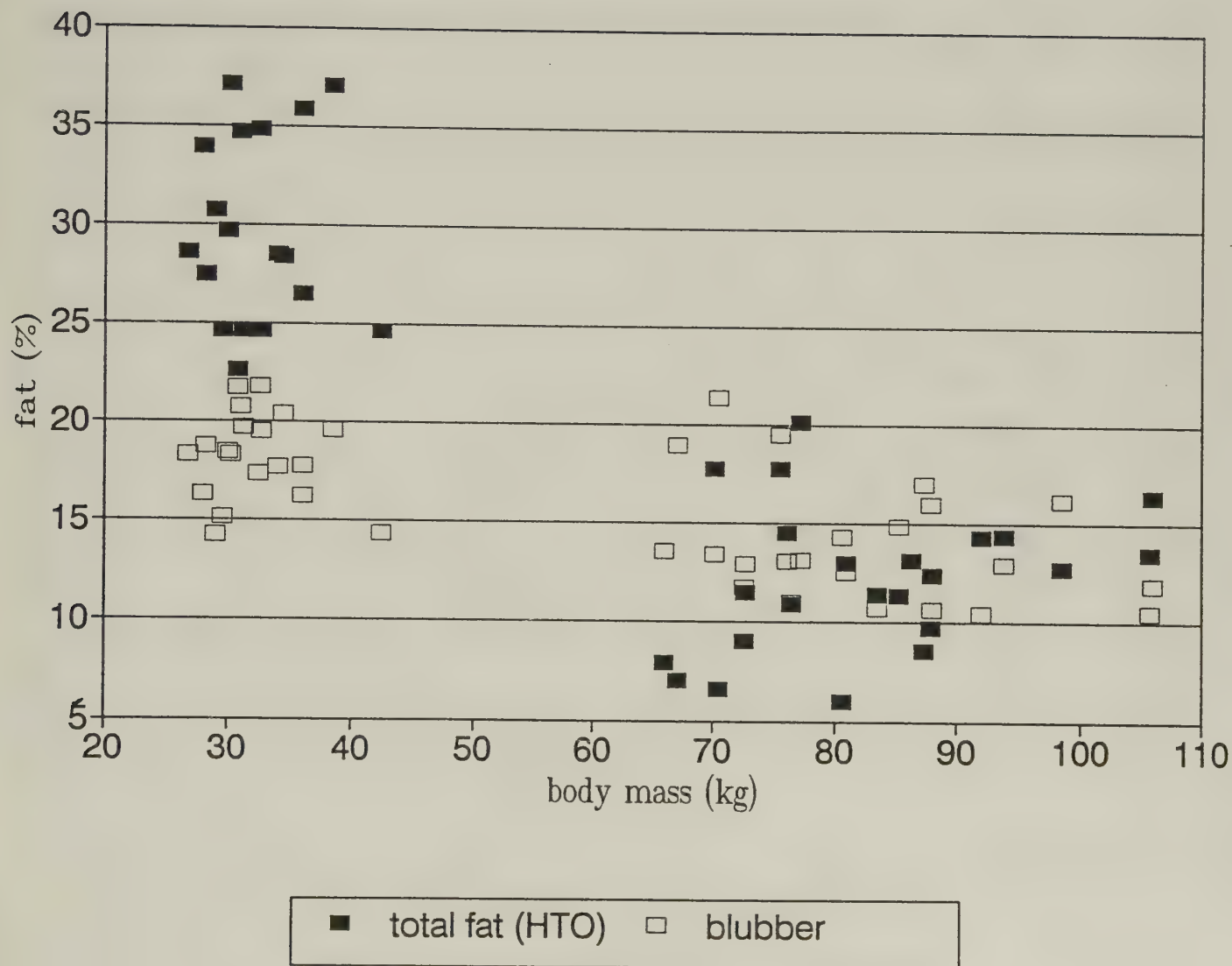


Figure 5: Total body fat (calculated by using tritiated water) compared to blubber mass (calculated from girth and blubber depth data) for California sea lions handled on San Nicolas Island in April 1990 and 1991.

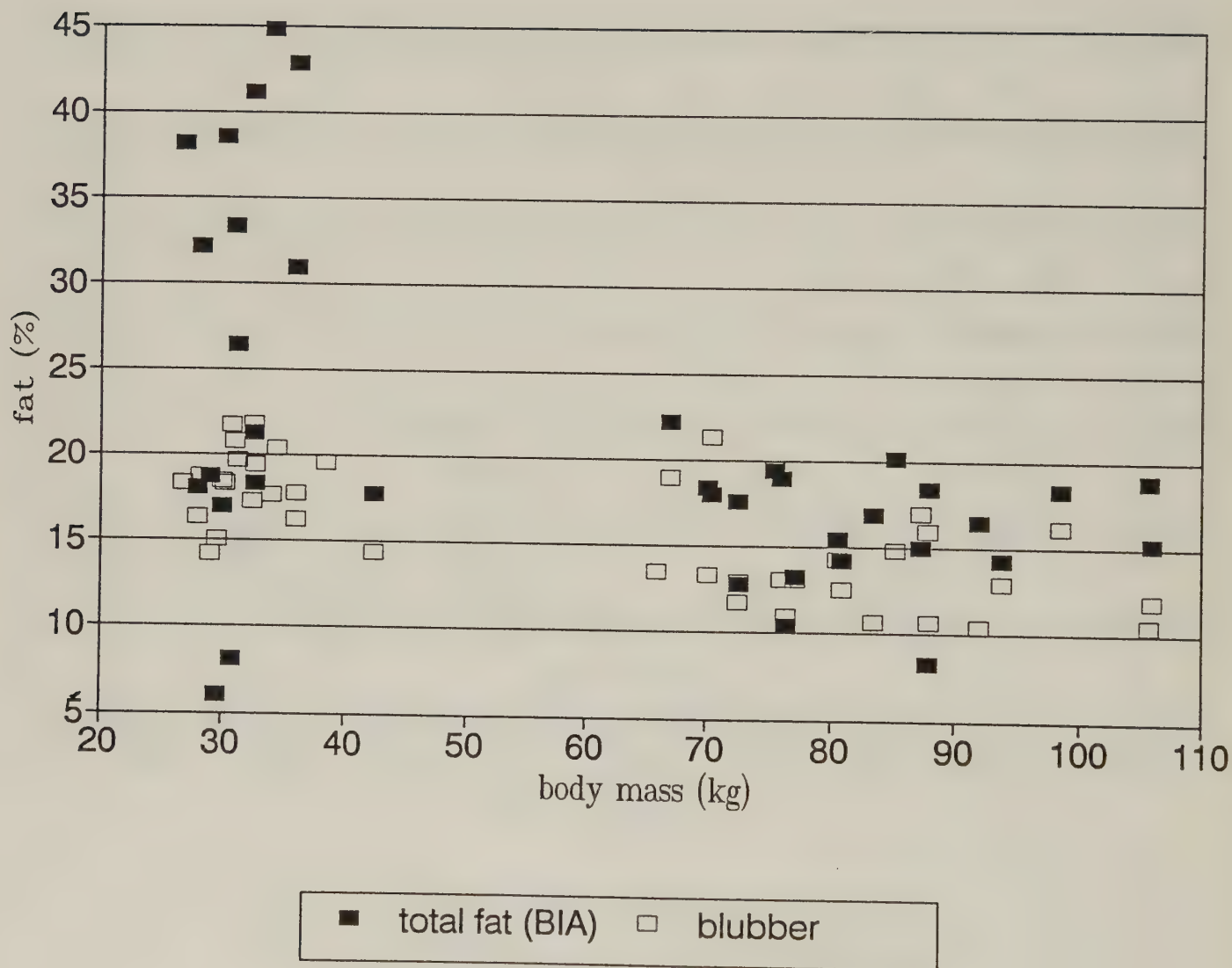


Figure 6: Total body fat (calculated by using BIA) compared to blubber mass (calculated from girth and blubber depth data) for California sea lions handled on San Nicolas Island in April 1990 and 1991.

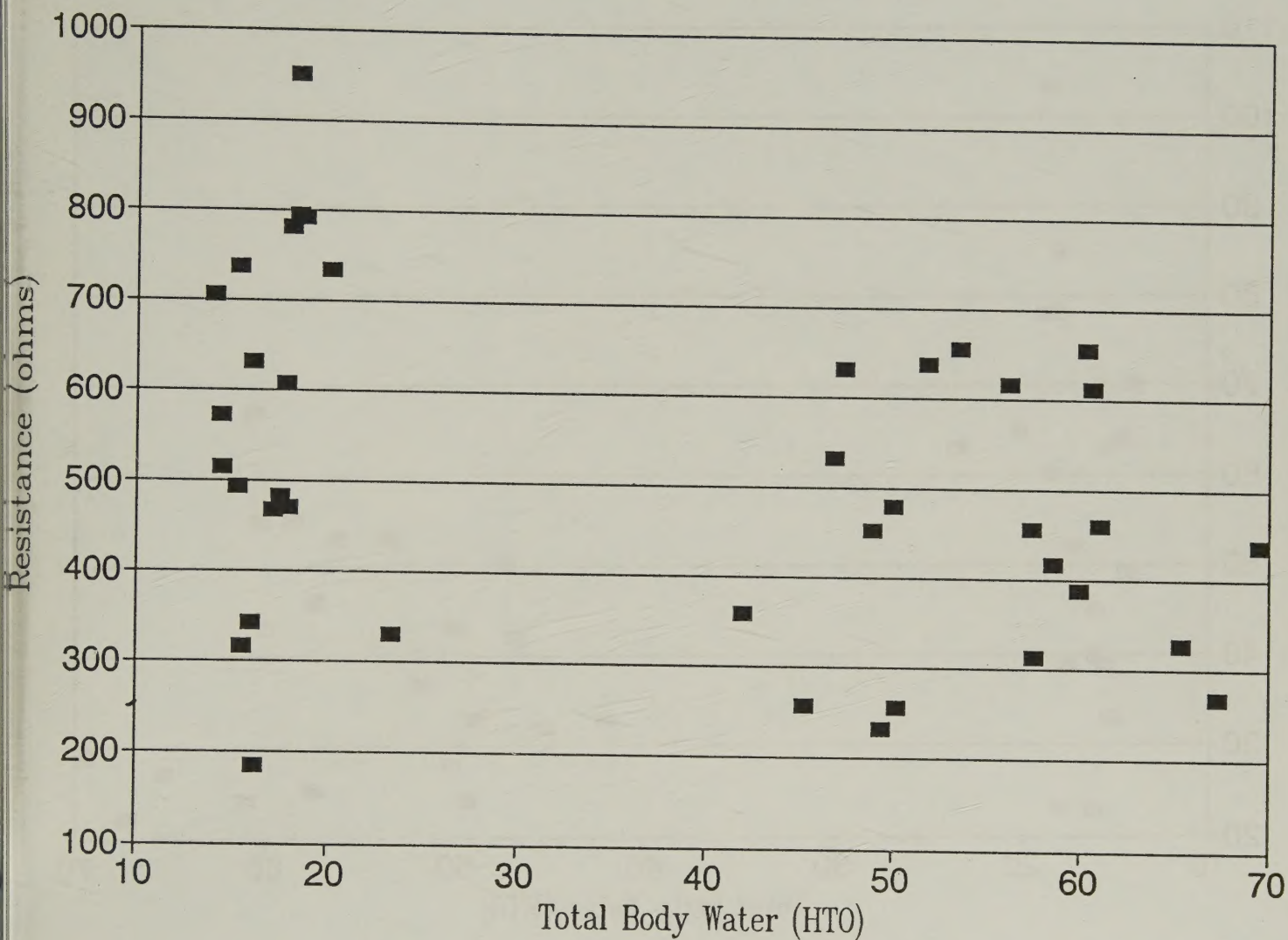


Figure 7: Resistance as a function of total body water (calculated from tritiated water dilution) for California sea lions handled on San Nicolas Island in April 1990 and 1991.

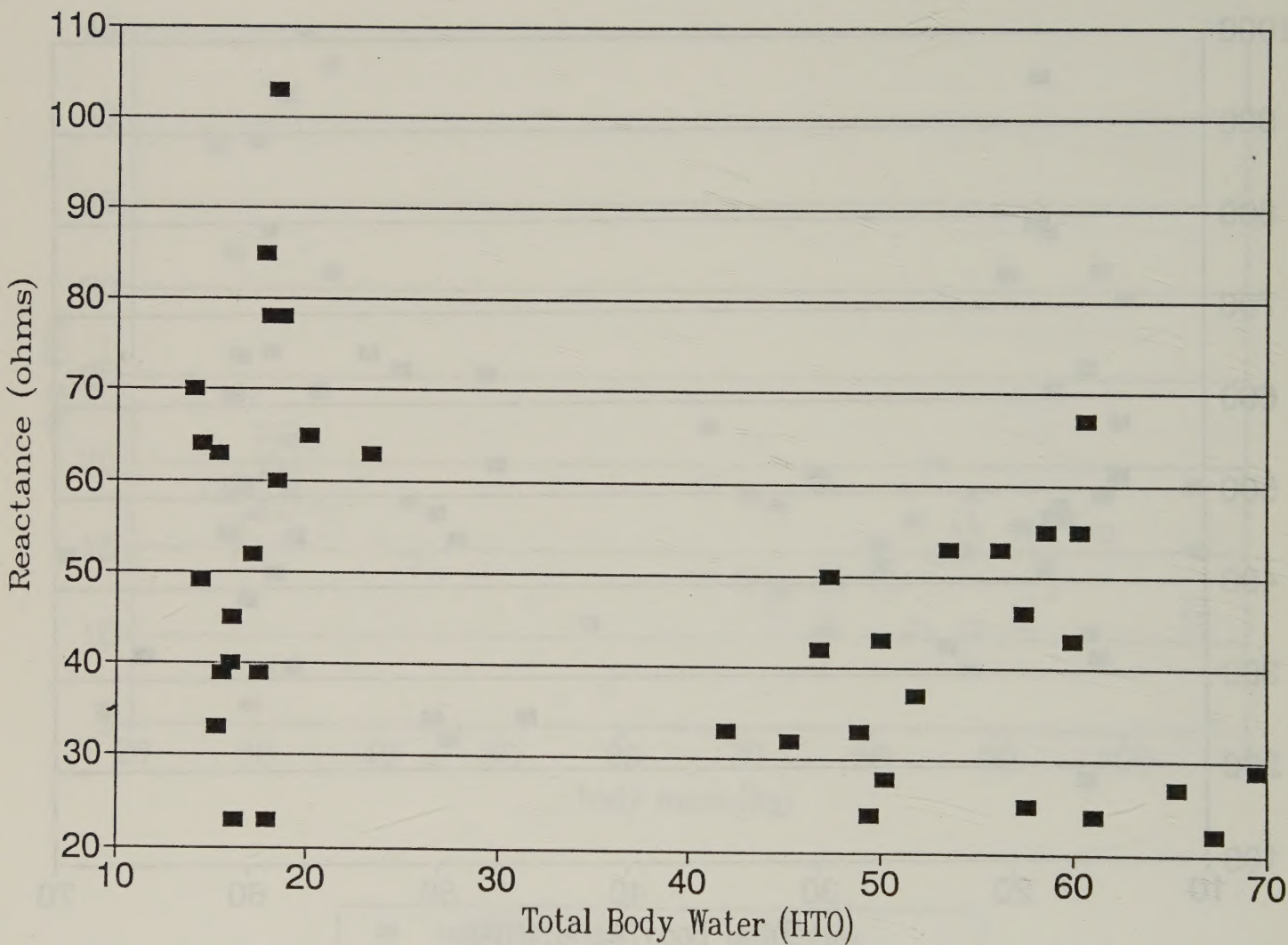


Figure 8: Reactance as a function of total body water (calculated from triitated water dilution) for California sea lions handled on San Nicolas Island in April 1990 and 1991.

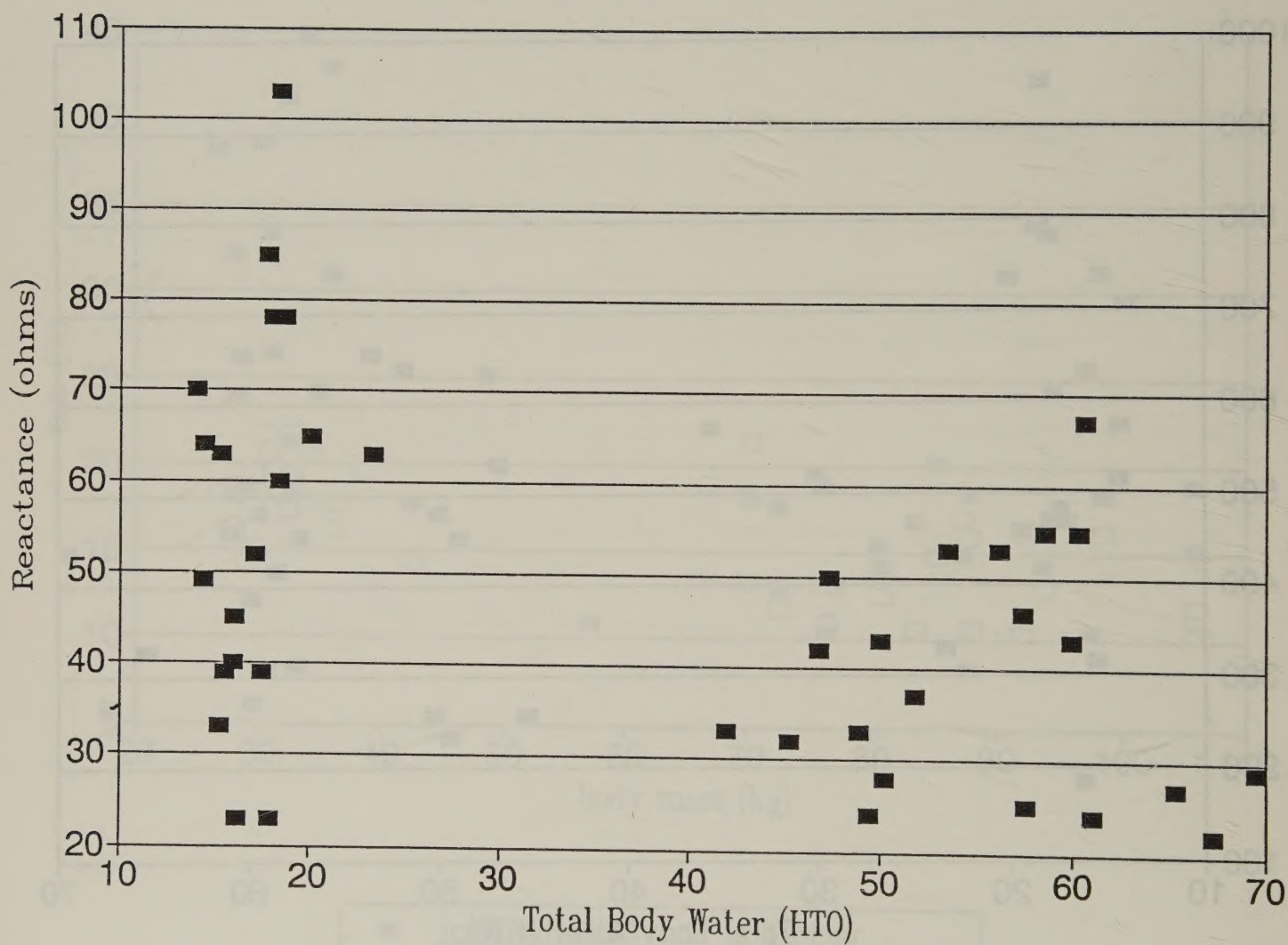


Figure 8: Reactance as a function of total body water (calculated from tritiated water dilution) for California sea lions handled on San Nicolas Island in April 1990 and 1991.